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Photodegradation of *Sodium Rifamicyn SV*

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ABSTRACT

The intense colouring antibiotic Sodium Rifamicyn SV *(Rfc) is decomposed by direct and sensitized photoirradiation, through a complex mechanism. This influences the microbiological activity of Rfc. It reacts from its electronic excited states upon light absorption and by a singlet molecular oxygen- and superoxide ion-mediated mechanism upon sensitized photoirradiation, employing Rose Bengal as a dye sensitizer. The rate constant for the reaction with singlet molecular oxygen was determined as* 1.2×10^8 *and* 5.1×10^8 $M^{-1}s^{-1}$ *at pH 6 and 12, respectively, by means of comparative methods. Both an increase in the decomposition rate as the solvent polarity decreases and a clear dependence of the decomposition kinetics on the pH of the medium can be observed. The latter factor suggests that the hydroquinonic structure of Rfc is the molecular moiety responsible for the photorreaction. The decrease in the antimicrobial power parallels the progress of the photodegradation. This indicates the lack of antibiotic activity by the reaction products.* © *1998 Elsevier Science Ltd*

Keywords: photodegradation, sensitized photoxidation, singlet molecular oxygen, sodium rifamicyn, superoxide ion.

INTRODUCTION

Solar or ambient light exposure of intense colouring photosensitive compounds can produce different types of chemical modifications, depending on

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the nature of the compound and the photoprocesses involved [1]. These events are of special relevance in substrates with pharmacological applications. The problem becomes critical in compounds for external use, because they can photodecompose after application, upon light irradiation, changing their therapeutic properties.

In this paper we report a study on the decomposition of the antibiotic *Sodium Rifamicyn SV* (Rfc), upon visible-light irradiation in solution. This compound is obtained from the chemical transformation of Rifamicyn B, a metabolic product of *Nocardia Mediterranei* [2]. It is widely employed in pharmacy as an antibiotic for external (topic) use [3]. The compound, whose structure is shown in Fig. 1, presents an intense electronic absorption in the visible region of the electromagnetic spectrum (Fig. 2).

Investigations were carried out in media of different pH, solvent polarity and aerobicity, the aim of the study being the elucidation of the kinetics and mechanism involved in the Rfc photodecomposition. In addition, through a simple microbiological monitoring, the evolution of the bacteriological power of the photoirradiated antibiotic was evaluated, in order to establish the extent of the photodegradation effect on the microbiological behaviour.

EXPERIMENTAL

Chemicals

Rfc was purchased from Sigma. The purity was checked by TLC, according to the methodology described in the Farmacopea Nacional Argentina [3] (Argentine National Pharmacopoeia). Sodium azide (NaN_3), furfuryl alcohol (FFA), Rose Bengal (RB) and superoxide dismutase (SOD) were purchased from Sigma. The buffers for pH 4–12 were prepared from Na_2HPO_4

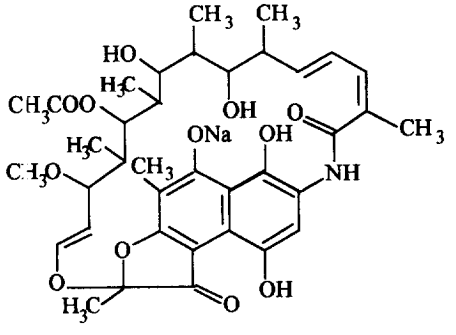

Fig. 1. Chemical structure of *Sodium Rifamicyn SV*.

and Na_2HPO_4 (both from Merck). Tridistilled water was employed. All solvents were of the highest purity available. Only freshly prepared solutions were utilized. Oxygen-free solutions were obtained by bubbling ultrapure N_2 (25 min) in the 1 cm path length absorption cuvette.

Methods

Absorption measurements were carried out with a Hewlett Packard 8452A diode array spectrophotometer. The progress of the decomposition in each solution was followed by a decrease in the 445 nm band.

The photolyzer was a PTI unit, provided with high pass monochromator and a 150 W Xe lamp. Irradiation was performed at 445 ± 10 nm and 548 ± 10 nm for the direct and RB-sensitized experiments. The electrode for oxygen uptake determinations has been described previously [4, 5].

Relative photodegradation rates and relative rates of oxygen uptake were determined from first order plots for substrate or oxygen consumption, as a function of irradiation time. In order to make the data straightforwardly comparable, the same initial number of quanta absorbed for Rfc and RB was employed in the respective series of direct and sensitized photolysis, respectively. Initial absorbances were 0.5 for Rfc at 445 nm and 1.5 for RB at 550 nm (the last one was higher in order to minimize the scanty absorption of Rfc).

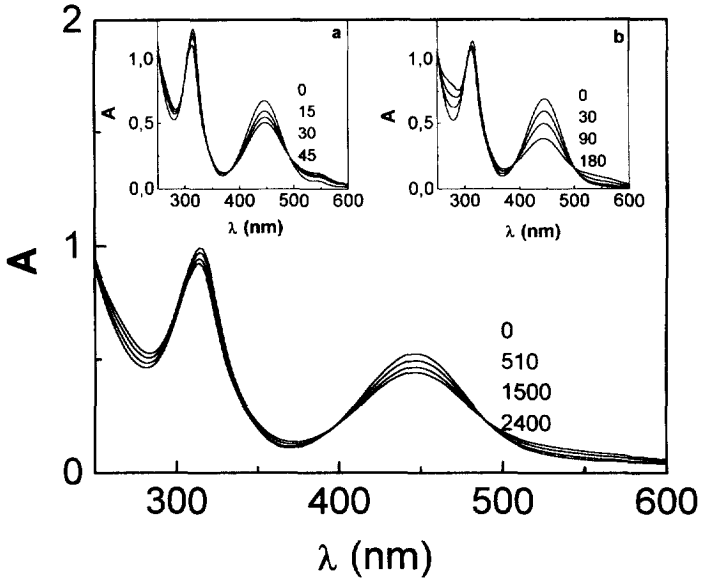

Fig. 2. Spectral evolution of Rfc upon direct photoirradiation (445 nm) at pH 12 (Rfc vs solvent). Insets: Spectral evolution of Rfc upon RB-sensitized irradiation (548 nm) at pH 12 (a) and pH 6 (b) (in both cases Rfc + RB vs RB). Numbers on the spectra denote irradiation time (s).

The determination of the rate constant k_r for the interaction of singlet molecular oxygen [$O_2(^1\Delta_g)$] with Rfc was performed employing the method of Foote and Ching [6], with FFA as a reference compound. Briefly, the method compares the slopes of the first order plots for substrate or oxygen consumption of independent photooxidation runs for the sample and a reference compound of known k_r, determined under identical experimental conditions.

The antibacterial activity of Rfc solutions was evaluated at different irradiation times, employing the Kirby–Bauer methodology [7], according to the Farmacopeia Nacional Argentina [3] and the norms of the document M2-A4 of the National Committee for Clinical Lab. Standards [8], using the standard stamp *Staphylococcus Aureus* (ATCC 25923). It was assayed in logarithmic phase on Petri's plate employing the Mueller–Hilton culture medium. The virgin disks for antibacterial test were generously provided by *Britania* (Argentina).

RESULTS

Preliminary assays demonstrate that, in dilute solutions (0.1 mM Rfc) the antibiotic presents a high sensitivity to polychromatic light under different experimental conditions.

Figure 2 shows the spectral evolution of Rfc direct photoirradiation in buffer pH 12 solution. Qualitatively similar changes were observed when the photolysis was performed in the absence of dissolved oxygen.

Two well defined isosbestic points can be observed in the spectra of Fig. 2. Difference absorption diagrams were linear, indicating that no absorbing intermediate was involved.

We also performed sensitized photolysis assays, in which a dye-sensitizer (RB) was added to the reaction vessel containing the Rfc solution. In this case practically all the light was absorbed by the sensitizer. The spectral changes are shown in Fig. 2, inset at two different pH values.

On the basis of the experimental evidence, the following reaction scheme was utilized in the analysis and discussion of the data.

$$\mathrm{Rfc} \xrightarrow{h\nu} \mathrm{Rfc}^* \xrightarrow{[O_2]} \begin{cases} \rightarrow \text{Prod. 1 (a)} \\ \rightarrow \text{oxidative species (b)} \end{cases} \tag{1}$$

$$\mathrm{Sens} \xrightarrow{h\nu} \mathrm{Sens}^* \xrightarrow{[O_2]} \text{oxidative species} \tag{2}$$

$$\text{oxidative species} + \mathrm{Rfc} \rightarrow \text{Prod. 2} \tag{3}$$

Rfc absorbs luminic radiation, generating its electronic excited states. From these states Rfc decomposes and/or generates oxidative species, in the presence of dissolved molecular oxygen (reactions (1a) and (1b), respectively). When a sensitizer (Sens) is present, constituted in our case by RB, the electromagnetic radiation is exclusively absorbed by it. Again, from the excited states of RB oxidative species can be produced (reaction (2)). Finally, the oxidative species can react with Rfc, and Prod. 2 are obtained (reaction (3)).

In order to establish the Rfc photodegradative mechanism we carried out a systematic study, employing different environmental conditions that could influence the photochemical behavior of an antibiotic of external application, as follows.

Direct photoirradiation

Rfc solutions at different pH values in the range 6 to 12 were irradiated. The respective photodegradation rates are significantly different, as shown in Table 1. The rates were identical when the runs were performed in the presence of additives such as NaN_3 (1 mM) or SOD (5 μg m^{-1}), well known quenchers [9] of $O_2(^1\Delta_g)$ and [10] superoxide ion ($O_2^{\cdot -}$) respectively. On the basis of the described experiments it can be assumed both the occurrence of reaction (1a) and the absence of reaction (3).

Effect of pH

The deprotonation equilibrium of the first –OH in the hydroquinone moiety [11] (pK = 10.35) of Rfc molecule (Fig. 1) suggests a possible effect of pH on the photodegradation rates of the antibiotic. We determined these rates at pH 4, 6, 8, 10 and 12, and the results are shown in Table 1. As can be seen, the photodegradation rate increases as pH increases. In addition, at two different pH values (6 and 10) direct photolysis of Rfc solutions in the presence and absence of dissolved oxygen rendered identical photodegradation rates for each pH value.

TABLE 1
Relative Photodegradation Rates of Rfc Buffered Aqueous Solutions as a Function of pH Upon Direct Irradiation

pH	*Relative rate*
4	0.083
6	0.27
8	0.57
10	0.98
12	1.00

Solvent polarity effect

Experiments of Rfc photolysis were made employing solvents of different dielectric constant [11] (ε): water ($\varepsilon = 80$); EtOH-Water 1:1 (v/v) ($\varepsilon = 52$); EtOH ($\varepsilon = 24$) and EtOH-toluene 1:1 (v/v) ($\varepsilon = 13$). The lower limit for the dielectric constant was determined by Rfc solubility. Results, shown in Fig. 3, exhibit a clear dependence of Rfc photodegradability with the polarity of the medium. The initial *quanta* absorbed by Rfc during photoirradiation in the different solvents was the same. The antibiotic photodecomposes much faster as the dielectric constant of the medium decreases, the relative (R) rate values being as follows: $R_{\text{EtOH-Tol}} = 3.5$ $R_{\text{EtOH}} = 5.2$ $R_{\text{EtOH-water}} = 10.4$ R_{water}.

Indirect (sensitized) photolysis

The UV spectra of Rfc (Fig. 2, insets) indicate that the antibiotic photodegradates in the presence of the xanthene dye RB. When 1 mM NaN_3 was added to the reaction vessel, no photorreaction could be observed. The inhibition by SOD (5 μg ml^{-1}) was only partial. These experiments constitute a demonstration of the occurrence of reaction (3), when the oxidative species are generated by RB (reaction (2), and the lack of Rfc as a generator of oxidative species (reaction (1b)). In the presence of additives, the kinetic effect was

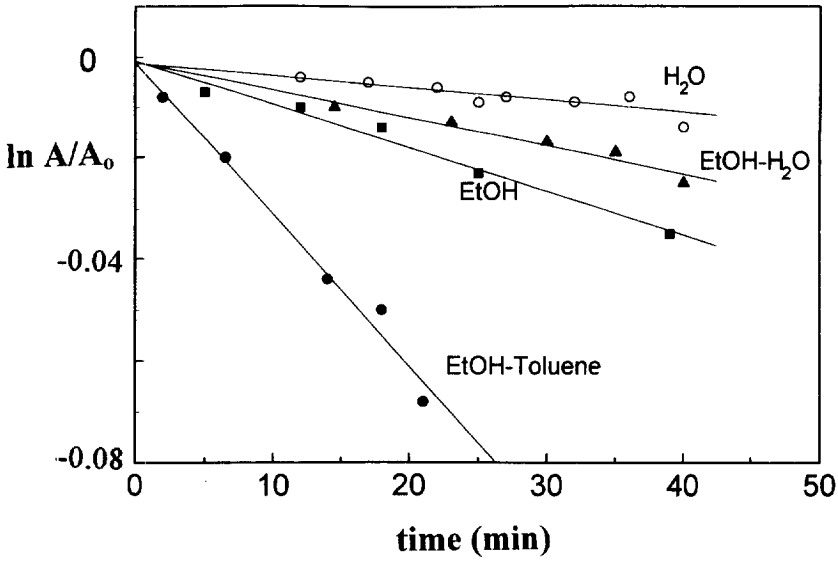

Fig. 3. First order plots for Rfc photodegradation in different solvents, upon direct photoirradiation. A and A_0 represent the absorbance at 445 nm of Rfc solutions at time $t = t$ and $t = 0$, respectively. The mixtures EtOH–H_2O and EtOH–toluene were 1:1 (v/v).

dependent on the concentration of the inhibitor, i.e. it was decreased when the concentrations of NaN_3 and SOD were reduced to one half. Besides, the rate of Rfc consumption was greatly increased (7 to 8 times faster) when the experiment was done in D_2O. This fact, in conjunction with the inhibitory effect of NaN_3 are currently accepted criteria [12] for the demonstration of the involvement of $O_2(^1\Delta_g)$ as an oxidative species. Nevertheless, the experiments in the presence of SOD also indicates a minor contribution of $O_2^{\cdot -}$ to reaction (3).

Rates of oxygen consumption

Using the specific oxygen electrode, we measured the rates of oxygen uptake by the photoirradiated Rfc solutions under several experimental conditions, as reported in Table 2 (reaction (3)).

Only the RB-sensitized photorreaction, at both pH 6 and 12, exhibited oxygen consumption. Neither Rfc nor RB alone, under direct irradiation, produced a measurable oxygen uptake. This property is well known for RB [13], when irradiated with moderate doses of visible light. For this reason RB is employed as a sensitizer in $O_2(^1\Delta_g)$ reactions. The quantum yield of $O_2(^1\Delta_g)$ generation by the dye in aqueous solution [14] is 0.74. It is also known [13] that RB generates approximately 70% $O_2(^1\Delta_g)$ and 25% superoxide ion ($O_2^{\cdot -}$). In the case of Rfc, the absence of oxygen consumption upon direct irradiation constitutes an additional demonstration that the antibiotic itself does not generate any of the reactive oxygen species. The rate of oxygen uptake in RB-Rfc solutions (at pH 7 and 10) was decreased by the presence of NaN_3 again in a concentration-dependent fashion. In order to quantify the kinetics of oxygen consumption by Rfc in the RB-sensitized experiments, we employed the already mentioned method of Foote and Ching [6]. The k_r value [10] for the reference compound (FFA) is 1.2×10^8 M^{-1} s^{-1} (reaction (4)). This value is independent of the pH [10]. The k_r values for Rfc (reaction (5)) resulted in (see Methods) 1.2×10^8 M^{-1} s^{-1} and 5.1×10^8 M^{-1} s^{-1} at pH 6 and 12, respectively (these values being upper limits for the rate constant, assuming that some contribution to oxygen uptake could be due to a $O_2^{\cdot -}$-mediated mechanism).

$$\text{FFA} + O_2(^1\Delta_g) \rightarrow \text{Prod. 3} \tag{4}$$

$$\text{Rfc} + O_2(^1\Delta_g) \rightarrow \text{Prod 2} \tag{5}$$

Microbiological analysis

The inhibitory microbiological activity of Rfc solutions before and after photolysis was evaluated at pH 7. Results are shown in Fig. 4. It can be

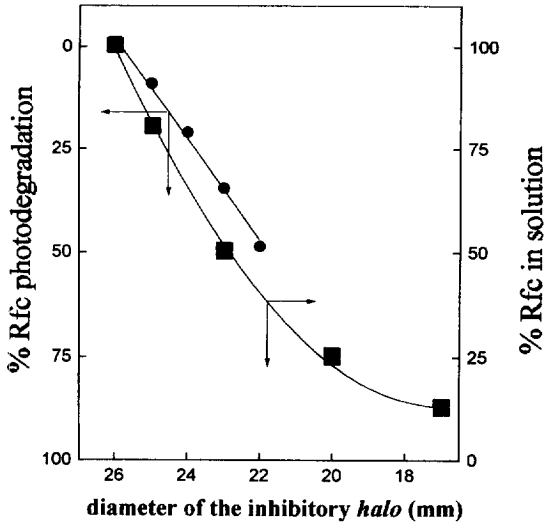

Fig. 4. Dependence of the antimicrobial activity of Rfc on Rfc concentration (right ordinate axis) and on the percent of Rfc photodegradation (left ordinate axis). Solutions of 0% photodegradation and 100% Rfc in solution had identical absorbance at 445 nm (concentration 25 mg l^{-1}).

seen that after 48.5% Rfc conversion, the inhibitory diameter decreases by about 15%. The corresponding calibration curve of Fig. 4 clearly shows the diminution of the inhibitory *halo* as the Rfc concentration decreases. The portion of the curve up to a 50% diminution in Rfc concentration shows a *quasi* parallel slope to that obtained for the photodegraded antibiotic.

TABLE 2
Photodegradation Rates of Rfc Upon Direct and RB-Sensitized Irradiation in the Presence of Different Additives in Aqueous Buffered pH 6 and pH 12 Solutions

Composition of the solution	*pH*	*Relative rates of oxygen consumption*
Rfc + RB	6	1
RB + met (0.5 mM)	6	0.13
Rfc + met (0.5 mM)	6	0
Rfc + RB + NaN_3 (2 mM)	6	0.21
Rfc	6	0
RB	6	0.007
Rfc + RB	12	1
RB + met (0.5 mM)	12	0.06
Rfc + met (0.5 mM)	12	0
Rfc + RB + NaN_3 (2 mM)	12	0.7
Rfc	12	0
RB	12	0

Regarding Fig. 4, it is important to point out that the absorbances at 445 nm for 0% photodegradation (left ordinate axis) and 100% Rfc in solution (right ordinate axis) were identical, and the respective decreases in concentration (% of photodegradation and % of Rfc in solution) were evaluated from the decreases in the 445 nm absorption maxima. For this reason, a certain interference due to products absorbance, especially in the advanced stages of the photorreaction, cannot be totally disregarded.

DISCUSSION

The evidences described earlier clearly indicate that Rfc is sensitive to photoinduced degradation under different experimental conditions. The mechanism governing this decomposition seems to be very complex. Nevertheless, in a first approach we can suggest three main reaction pathways.

The first one is represented by the decomposition of Rfc from its electronic excited states upon light absorption. This mechanism is not connected to any of the reactive oxygen species such as $O_2(^1\Delta_g)$ or $O_2^{\cdot -}$, as demonstrated by the absence of inhibition by specific scavengers for the mentioned oxidative species.

The solvent polarity effect on the photolitic process suggests a loss of polarity in the Rfc-photoproducts as compared to the original structure. The kinetic behavior as a function of pH in the direct photoirradiation experiments (Table 1) clearly indicates that the ionization of the hydroquinonic moiety plays an important role, even when the photodegradative mechanism operated *via* non-oxygenated pathways.

The second and third mechanisms take place during the RB-sensitized photolysis. They involve the participation of $O_2(^1\Delta_g)$ and $O_2^{\cdot -}$ as oxidative agents for Rfc phototransformation. The spectral evidence indicates that the products generated by both mechanisms are different from those produced by direct photoirradiation. The values obtained for k_r are in the order of those expected for a $O_2(^1\Delta_g)$ mediated process in phenolic compounds [15]. Furthermore, the increase of k_r with pH constitutes an established characteristic for the photooxidative behaviour of phenols and hydroxybenzenes, including hydroquinone [5, 15, 16]. These experimental evidences constitute strong arguments in favor of the involvement of the hydroquinonic structure of Rfc in the photooxidative pathway.

Finally, the microbiological experiments indicate a clear decrease of the activity in photolyzed Rfc. The parallelism between the antimicrobial behaviour in photolized Rfc solutions and non-photolized solutions of decreased Rfc concentration lead us to infer that the photoproducts of Rfc degradation does not possess any activity against the *staphylococcus aureus* employed in our tests.

The experimental data presented in this paper have relevance to the prevention of photoinduced loss of Rfc antimicrobial activity. They draw attention to storage conditions of Rfc, and possible photodegradation of the antibiotic, upon topic application as a medicine, followed by solar exposure.

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